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Microhabitat Use Affects Brain Size and Structure in Intertidal Gobies

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ABSTRACT

The ecological cognition hypothesis poses that the brains and behaviours of individuals are largely shaped by the environments in which they live and the associated challenges they must overcome during their lives. Here we examine the effect of environmental complexity on relative brain size in 4 species of intertidal gobies from differing habitats. Two species were rock pool specialists that lived on spatially complex rocky shores, while the remainder lived on dynamic, but structurally simple, sandy shores. We found that rock pool-dwelling species had relatively larger brains and telencephalons in particular, while sand-dwelling species had a larger optic tectum and hypothalamus. In general, it appears that various fish species trade off neural investment in specific brain lobes depending on the environment in which they live. Our previous research suggests that rock pool species have greater spatial learning abilities, enabling them to navigate their spatially complex environment, which may account for their enlarged telencephalon, while sand-dwelling species likely have a reduced need for spatial learning, due to their spatially simple habitat, and a greater need for visual acuity. The dorsal medulla and cerebellum size was unaffected by the habitat in which the fish lived, but there were differences between species indicative of species-specific trade-offs in neural investment.

Introduction

The habitat in which animals operate exerts profound selective pressures on their brains and behaviours, such that they are exquisitely adapted to the habitat in which they live [Sherry et al., 1992]. Trying to attribute evolutionary adaptations to specific contemporary forces of natural selection, however, can be fairly difficult. For example, the structure of the vertebrate brain has been altered greatly throughout evolutionary history, but it can be difficult to identify exactly what contemporary selective forces have played a role in shaping this structure and which of its characteristics are actually adaptations and which are in fact caused by differences in phylogeny (i.e. the ghost of selection past).

During the course of evolution, changes occurring in the physical environment have been shown to run parallel with specialized changes in an animal's behavioural patterns, external characteristics, brain morphology and life history strategy. Numerous studies conducted on a wide range of taxa have shown that the size of certain brain areas is correlated with ecological conditions in birds [Healy and Guilford, 1990], reptiles [Day et al., 1999], mammals [Galea et al., 1996; Shultz and Dunbar, 2006] and fish [Huber

et al., 1997]. Furthermore, a general hypothesis has been generated to explain these brain/habitat correlations. The ecological cognition hypothesis suggests that the brains and behaviours of individuals are largely shaped by the environments in which they live and the associated challenges they must overcome during their lives [Healy and Braithwaite, 2000]. Central to this hypothesis is the assumption that maintenance, operation and production of the neural machinery required for learning and memory is energetically costly and thus should only be selectively invested in by animals that have an ecological demand for it [Dukas, 1999]. For example, larger brains have evolved much more readily in active predators than in grazers, indicating that they require a greater neurobiological capability to strategically search for their prey [Parker and Gibson, 1977; Striedter, 2005]. Furthermore, larger brains have been correlated with greater learning abilities but smaller guts and a lower offspring production in guppies [Isler, 2013; Kotrschal et al., 2013], greater parental investment but smaller guts in cichlids [Tsuboi et al., 2014], a greater learning ability but shorter adult longevity and a lower larval competitive ability in *Drosophila* [Mery and Kawecki, 2003; Burger et al., 2008; Kolss and Kawecki, 2008] and greater cognitive performance but reduced annual fertility rates and the development of cooperative breeding in mammals [Isler and van Schaik, 2009].

In order to gain a better understanding of the role ecological forces play in shaping vertebrate brain evolution, it is common practice to carry out analyses on closely related species that inhabit a diverse range of habitats. Fish are an ideal taxonomic group for such studies. The vast number of species and their ability to live in a diverse range of habitats exposes fish to varying degrees of selection for specialized behaviours and sensory abilities, which in turn have impacted brain evolution [Ullmann et al., 2010]. As a consequence, an enormous range of brain morphologies has evolved in fish [Kotrschal et al., 1998; Nieuwenhuys and Meek, 1998]. Despite the diversity of fish brains, they are still comparable to those of other vertebrates in terms of function and morphology [Wullimann and Mueller, 2004; Broglio et al., 2011]. The fish brain consists of 7 main structures: olfactory bulbs, the telencephalon, the optic tectum, the cerebellum, the dorsal medulla, the pituitary and the hypothalamus, with a number of centres for higher processing homologous to other vertebrate brain regions [Broglio et al., 2003; Ullmann et al., 2010]. Thus, it should come as no surprise that the correlations between brain size and environmental complexity observed in other vertebrates have also been observed in fish. For example, comparative studies on African cichlids have linked variations in telencephalon, cerebellum and olfactory bulb size to environmental complexity, optic tectum size to diet and water depth, hypothalamus size to mating strategy and dorsal medulla size to trophic position and mating competition [Van Staaden et al., 1995; Huber et al., 1997; Pollen et al., 2007; Gonzalez-Voyer et al., 2009a, b; Gonzalez-Voyer and Kolm, 2010].

The telencephalon is greatly involved in spatial learning in fish [Salas et al., 2003], and differences in telencephalon morphology may correlate with differences in ecological demands for spatial learning [Odling-Smee et al., 2006]. Numerous studies have found larger telencephalons in mobile fish foraging within spatially complex environments compared to fish that forage in less complex environments [Huber et al., 1997; Kotrschal et al., 1998; Pollen et al., 2007; Gonda et al., 2009, 2011; Costa et al., 2011]. Controlled laboratory studies have demonstrated that salmon [Kotrschal et al., 2012; Näslund et al., 2012; Salvanes et al., 2013] and zebra fish [von Krogh et al., 2010] show an increased telencephalon size when raised in spatially enriched environments compared to barren environments. Similarly, in Azorean rock pool blennies *(Parablennius sanguinolentus)* the variation in telencephalon size appears to be closely related to the spatial challenges associated with structurally complex environments [Costa et al., 2011]. Males of this species establish nests and remain within their nest area for the entire breeding season, whereas females travel large distances to spawn with males at different nest sites [Carneiro et al., 2001]. Thus, females have a greater need to remember specific locations of previously visited nest sites and to retain a spatial map of the area than do males, and hence they have a larger dorsolateral region compared to their telencephalon [Carneiro et al., 2001; Costa et al., 2011].

The goby family contains the most species of all marine fish (over 2,600 species in 230 genera), which live in a wide variety of habitats. Thus, it is surprising that only one study has explored the links between overall brain size and the environment in this family [Bauchot et al., 1989]. Here we compare the brain morphology of 4 of the most abundant goby species found on temperate rocky shores and sandy beaches in the Sydney region of Australia. These comprise the rock pool-dwelling species Cocos frillgoby *(Bathygobius cocosensis)* and Krefft's frillgoby *(B. krefftii),* and the sand-dwelling species eastern longfin goby *(Favonigobius lentiginosus)* and Hoese's sandgoby *(Istigobius hoesei).* These two groups show extensive behavioural, habitat and life history differences [Thacker and Roje, 2011; White and Brown, 2013, 2014a] (see online suppl. figure; for all online suppl. material, see www.karger.com/doi/10.1159/000380875). Most importantly, rock pool-dwelling gobies remain within the intertidal zone during low tide by seeking refuge from these adverse conditions in specific home pools, whereas sand-dwelling goby species tend to follow the tide as it moves in and out. Our previous examination of their spatial learning capabilities showed that rock pool species have a high site fidelity, return to their home pools after displacement and out-perform sand-dwelling species in every task [White and Brown, 2013, 2014a–c].

Using these species, we addressed two primary questions: (1) do intertidal goby species differ in brain size and morphology? (2) Is brain morphology correlated with differences in ecology? According to the ecological cognition hypothesis, we predicted that natural selection would favour the evolution of larger brain structures associated with spatial learning (i.e. telencephalon) in species that live in spatially complex rock pool habitats. By comparison, the level of selective pressure on telencephalon size is expected to be significantly less in species that live in homogenous sandy habitats.

Methodology

Study Animals

Four intertidal gobies species (n = 10 for each species) were captured at low tide from a number of beaches and rock platforms along a 14-km stretch of coastline in Sydney, N.S.W., Australia. The 20 rock pool-dwelling gobies were collected from their home pools on 5 different rock platforms using small hand-held nets. The 20 sand-dwelling gobies were collected via a snorkel and hand nets from the soft sediment substrate of 4 different bays at a water depth of 1–3 m.

Specimen Preparation

Goby specimens were euthanized with an overdose of tricaine methane sulfonate (MS222) and their total length (L T : distance from the tip of the snout on the upper jaw to the end of the tail) and body weight were measured. Fish specimens were immediately placed under a dissecting microscope in a bath of distilled water, and their brains were exposed dorsally by removing the top of the neurocranium (i.e. frontal, parietal and nasal bones and in some cases the operculum). The specimens were then placed in a solution of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) buffer pH 7.2 to fix tissues for 2 days. After the fixation was complete, we rinsed them 3 times in 0.1 M PBS buffer, waiting 15 min between rinses. The brains were then removed completely from the neurocranium under a dissecting microscope by severing the cranial nerves using fine forceps, and the spinal cord was severed between the 2nd and 3rd vertebrae from the base of the neurocranium using a scalpel to ensure that the posterior hindbrain remained intact. We left the eyes attached to the brain to avoid severing optic nerves and to allow the brains to be easily picked up and moved without being damaged. Between imaging and histological analysis, brains were stored in 0.1 M PBS buffer at 4 °C.

Sectioning and Volumetric Analysis

All brains were stained in a solution of 1% methylene blue, 0.6% sodium bicarbonate and 40% glycerol (diluted with water to a ratio 1:1) for 2 min, embedded in agarose and stored overnight in 0.1 M PBS buffer at 4 °C. Brains were sliced into a series of 100-μm-thick sections on a Leica VT 1000S vibratome and mounted on a microscope slide (4 per slide) or stored in a well plate containing 0.1 M PBS to avoid dehydration until the imaging was complete. Sections were photographed immediately using a digital camera (Color-View IIIu) attached to an Olympus SZX16 research stereo microscope. The cross-sectional area of each brain lobe, i.e. the telencephalon optic tectum, the cerebellum, the hypothalamus and the dorsal medulla, was traced digitally (fig. 1) and analysed using analySIS docu version 5.0 (Olympus Soft Imaging Solutions, 2007). The brain lobe structural boundaries used in this study closely followed published descriptions by Davis and Northcutt [1983] and Wullimann et al. [1996]. Once the digital tracing was complete, the total volume of each lobe was calculated by adding the areas of all of the traced sections and multiplying them by the section thickness (100 μm) [Rosen and Harry, 1990; Costa et al., 2011]. The entire brain was also traced digitally from sections and the total brain volume was calculated by adding the areas of all of the traced sections and multiplying them by the section thickness.

Statistical Analysis

A factor that complicates brain volume comparisons is that brain size exhibits an allometric relationship with body size [Striedter, 2005; Deaner et al., 2007; Gonzalez-Voyer et al., 2009a, b]. Brain size has a strong positive correlation to body size, brain/body size ratios decrease as body size increases, and brain size has a tendency to show a stronger increase with increasing body size during early ontogeny rather than adulthood [see Striedter, 2005, for a detailed review of these brain-body size relationships]. To control for these allometric effects, we calculated the relative lobe volume for each fish specimen [Bullmore et al., 1995; Burish et al., 2004; Wiper et al., 2014]. For example, when examining the relative lobe volume, body size was controlled for by dividing the lobe volume by the total brain volume to give the relative value (i.e. telencephalon volume/total brain volume = relative telencephalon volume). Furthermore, all fish specimens used in this study were large (>4 cm) to ensure that the variation in brain/body size was limited between specimens within the same species. Nevertheless, we cannot rule out the possibility that the relative size of different brain regions may vary with age, even if animals have the same body length. To test if total brain size was affected by habitat type or species differences (while controlling for body size), we used an ANCOVA design with total brain volume as the dependent variable, sex and habitat or species as factors and body size (total length) as a covariate. For the brain structure analyses, we conducted 2 MANOVAs including the relative volumes of all 5 brain lobes (telencephalon, optic tectum, hypothalamus, cerebellum and dorsal medulla) as dependent variables and sex and habitat or species as factors. After establishing significant effects of both habitat and species, we performed ANOVAs for each brain lobe separately to determine the effects of habitat and species on each of the brain lobes. Further ANOVAs were performed on each brain lobe separately and split by species to determine the effects of sex on lobe size. We expected that some lobes (e.g. the telencephalon) would differ between habitats while others (e.g. the dorsal medulla) would not. All analyses were performed using StatView version 5.0.1 (SAS Institute Inc., 1998).

Results

Digital images of the brains from the 4 goby species studied showed that, although there is a degree of interspecific variability in brain morphology (fig. 1), the most substantial differences appear to exist between fish from different habitat types (i.e. rock pools and sand).

Fig. 1. Brains of 4 species of intertidal gobies, illustrating the interspecific variation in the relative size of the telencephalon (outlined in pink), the optic tectum (outlined in yellow), the cerebellum (outlined in green), the hypothalamus (outlined in blue) and the dorsal medulla (outlined in red) (colours refer to the online version only). Cocos frillgoby *B. cocosensis* (ai–av), Krefft's frillgoby *B. krefftii* (bi–bv), eastern longfin goby *F. lentiginosus* (ci–cv) and Hoese's sandgoby *I. hoesei* (di–dv). a–d Lateral views of the brains. a–di Sections through the telencephalon. a–dii Sections through the telencephalon, optic tectum and hypothalamus. a–diii Sections through the optic tectum, cerebellum and hypothalamus. a–div Section through the optic tectum, cerebellum and hypothalamus. a–dv The approximate position of each of the sections (i–v) for all species is illustrated by the white dotted lines in the lateral view of the brain in a . Scale bars range from 200 µm to 2 mm and are depicted on the relevant images.

Table 1. Taxonomic information, habitat locality, mean body length, and mean brain volume for the 4 goby species investigated in this study

Species	Common name	Habitat	Mean body length, cm	Mean brain volume, mm^3
B. cocosensis	Cocos frillgboy	Rock pool	4.61	9.41
B. krefftii	Krefft's goby	Rock pool	4.64	9.26
F. lentiginosus	Eastern longfin goby	Sand	4.55	7.81
I. hoesei	Hoese's sandgoby	Sand	4.89	10.80
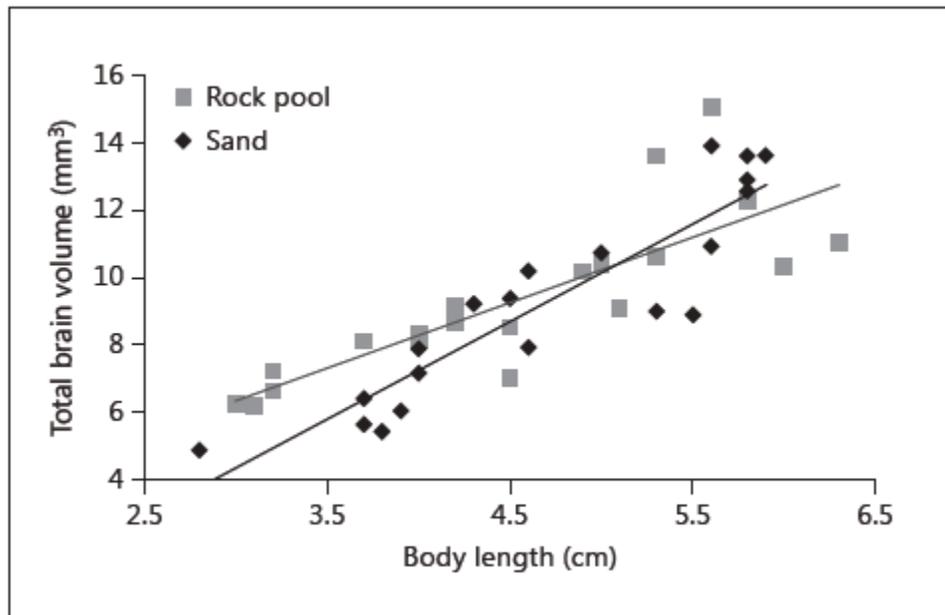
Fig. 2. Allometric relationships between body size and brain size for rock pool-dwelling (y = 1.9318x + 0.5529, R^2 = 0.6676) and sand-dwelling (y = 2.9x – 4.3537, R^2 = 0.8291) goby species.

The 4 goby species studied exhibited variation in absolute brain volume and body size (table 1). Analyses of absolute brain size detected a strong effect of habitat type but no effect of species (ANOVA: habitat, $F_{1,36}$ = 5.33, p = 0.027; species, $F_{3,32}$ = 1.409, p = 0.258). Fish living in rock pool habitats had slightly larger brains than fish from sand (rock pool fish mean size: 9.33 mm 3; sand fish mean size: 9.30 mm^3). We also found that the covariate body size was a strong predictor of brain size (ANOVA: habitat, $F_{1,36}$ = 115.61, p < 0.0001; species, $F_{3,32}$ = 112.54, p < 0.0001; fig. 2). Furthermore, the relationship between body size and brain size showed variation between rock pool species and sand species (habitat-body size interaction: $F_{1,36}$ = 4.642, p = 0.038). When analysing the total brain size at the species level, no significant differences were detected when controlling for body size (species-body size interaction: $F_{3,32}$ = 1.602, p = 0.208). Furthermore, regressions exploring correlations between lobe size and total brain size demonstrated that all lobes increase positively with brain size, displaying significant results (p = 0.05 in all cases) and high R 2 values; ratios were consistent across all species (see online suppl. table).

Quantitative analysis of the relative volumes of the 5 brain areas (telencephalon, optic tectum, cerebellum, dorsal medulla, and hypothalamus) supported the impressions gained via visual inspection of the brain morphology, i.e. that there are major differences in brain morphology between rock pool- and sand-dwelling species (fig. 3). The 2 MANOVAs found highly significant effects of habitat type ($F_{1,36}$ = 23.015, $p < 0.001$) and species ($F_{3,32}$ = 12.541, $p < 0.001$) on the relative size of the various brain lobes. Each area of the brain was then examined in isolation.

As predicted, rock pool-dwelling species had significantly larger telencephalons than sand dwellers ($F_{1,36}$ = 70.319, $p < 0.001$), accounting for 21% of the total brain volume compared to 13% in sand species. Of these, the Cocos frillgoby had the largest telencephalon, followed closely by Krefft's goby and then the sand species Hoese's sandgoby and the eastern longfin goby ($F_{3,32}$ = 40.613, $p < 0.001$) (fig. 4). Sex did not affect telencephalon size in any of the 4 species studied ($p > 0.1$ in all cases).
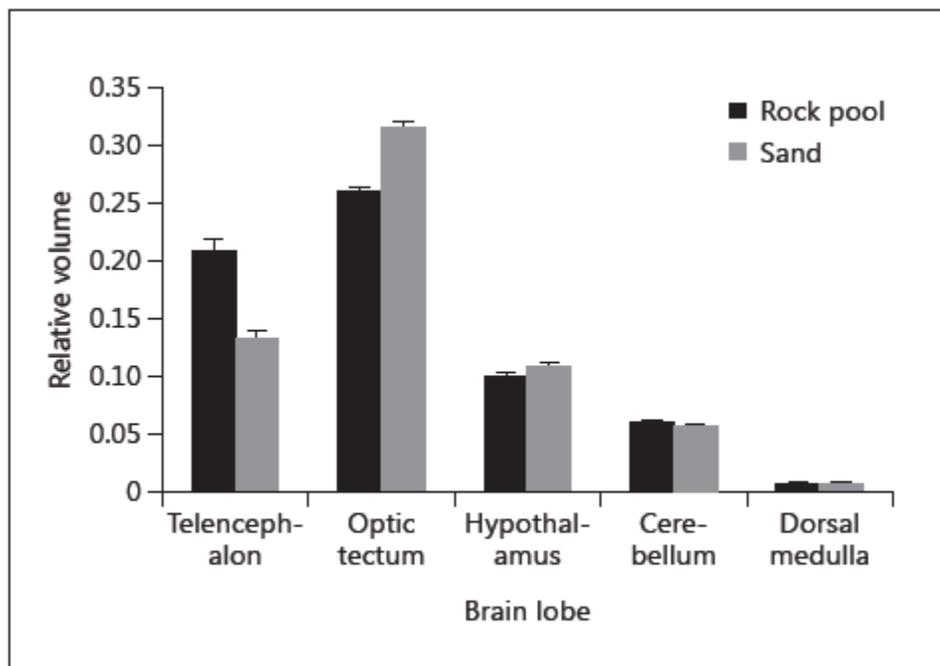

Fig. 3. Comparison of relative brain lobe volumes (mean ± SE) between the 2 habitat types.

The optic tectum was the largest brain area in all 4 species. This brain structure was significantly larger in the 2 sand-dwelling species ($F_{1,36}$ = 70.7, $p < 0.0001$), occupying 32% of the total brain volume. The rock pool species, in contrast, had a smaller optic tectum, accounting for 26% of the total brain volume. There were significant differences between species in terms of optic tectum size ($F_{3,32}$ = 21.305, $p < 0.001$). The optic tectum was particularly large in the eastern longfin goby, followed by Hoese's sandgoby and then Krefft's goby and the Cocos frillgoby (fig. 4). Sex did not influence optic tectum size in 3 of the 4 species ($p > 0.1$ in all cases). However, the rock pool-dwelling species Krefft's goby approached significance ($F_{1,8}$ = 3.816, $p = 0.086$); females tended to have larger optic tectums than males.

Cerebellum relative volume was not affected by habitat type ($F_{1,36}$ = 0.441, $p = 0.608$). However, there were significant differences at the species level ($F_{3,32}$ = 10.614, $p = 0.001$). The eastern longfin goby had

a significantly smaller cerebellum than all other species except for Krefft's goby [Fisher's protected least significant difference (PLSD), p < 0.001], and the Cocos frillgoby had a significantly larger cerebellum than all species except for Hoese's sandgoby Fisher's PLSD, p ≤ 0.0006). Sex did not control cerebellum size for any of the 4 species studied (p > 0.2 in all cases).
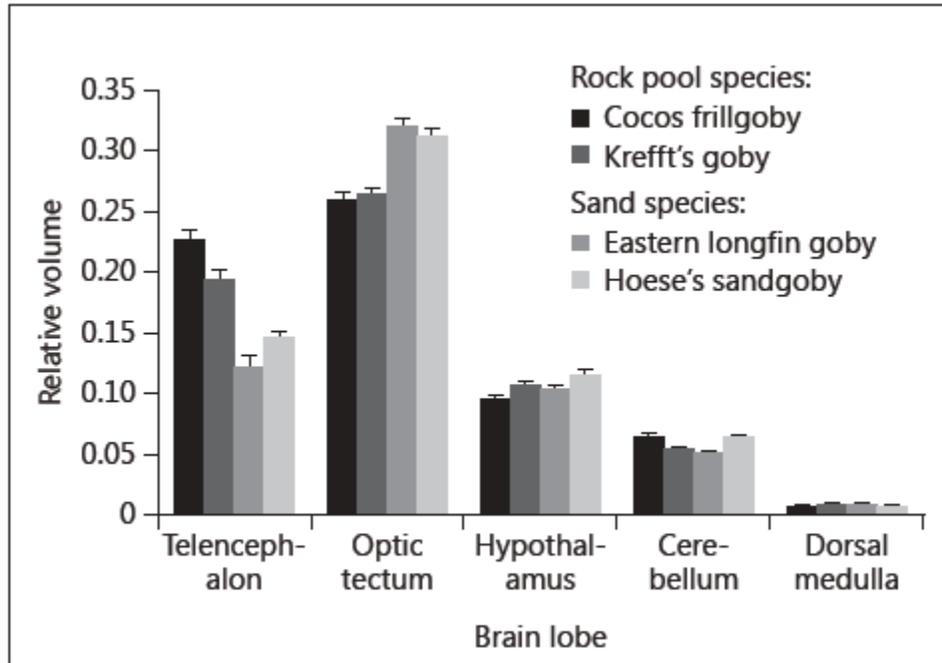

Fig. 4. Comparison of relative brain lobe volumes (mean ± SE) between the 4 goby species studied.

Sand-dwelling species had a significantly larger hypothalamus than rock pool-dwelling species ($F_{1,36}$ = 4.968, p < 0.032), accounting for 11% of the total brain volume (compared to 10% in rock pool-dwellers). Further analyses at the species level revealed that relative hypothalamus volume differed significantly between species ($F_{3,32}$ = 4.97, p < 0.006). The rock pool-dwelling species Cocos frillgoby had a significantly smaller hypothalamus than all other fish species (Fisher's PLSD, p < 0.05 in all cases), accounting for only 10% of the total brain volume (compared to 11–12% for the other species; fig. 4). Sex did not affect relative hypothalamus size for any of the 4 species studied (p > 0.2 in all cases).

Overall, the relative dorsal medulla volume was not affected by the habitat in which the fish lived ($F_{1,36}$ = 0.269, p = 0.607). However, there were significant species differences ($F_{3,32}$ = 3.659, p = 0.023). Krefft's goby had a significantly larger dorsal medulla (1% total brain volume; fig. 3) than all other species except for the eastern longfin goby (Fisher's PLSD, p < 0.01), and the dorsal medulla of the Cocos frillgoby (0.7% total brain volume) was significantly smaller than in all species except for Hoese's sandgoby (Fisher's PLSD, p < 0.059). Sex did not affect relative dorsal medulla size for any of the 4 species studied (p > 0.3 in all cases).

Discussion

The brain morphology of gobies from the two vastly different habitats exhibited significant variation in brain structure. While sand-dwelling gobies had a larger body size on average, their brains were considerably smaller than those of rock pool-dwelling species when controlling for body size. When brain

lobes were analysed separately, we found that the relative size of the telencephalon, the optic tectum, and the hypothalamus were correlated with variation in the environment from which they came. In contrast, the relative size of the cerebellum and dorsal medulla was largely independent of environmental variation, although there were differences between species. The differences and similarities observed in the patterns of brain evolution in individuals occupying distinctly different habitats contribute valuable insights into the way the nervous system functions when subjected to various environmental conditions in response to natural selection.

Rock pool-dwelling fish developed noticeably larger brains than sand species, both in literal terms and when body size was controlled for. In general, large-brained teleosts most often occur in spatially complex habitats, which has led to the suggestion that the evolution of brain size is influenced by the need to learn the complicated spatial arrangement of such environments and their inhabitants [Bauchot et al., 1977, 1989; Huber et al., 1997]. It certainly appears as though the rock pool-dwelling species devote significantly more energy to the development and maintenance of the brain (especially the telencephalon) compared to their sand-dwelling counterparts. Our previous studies suggest that at least some of this enhanced cognitive ability is devoted to spatial learning and homing behaviour in particular [White and Brown, 2013, 2014a–c].

In addition to the noticeable brain size differences between rock pool dwellers and sand dwellers, there was a significant variation in the relative volume of specific brain structures. As predicted, gobies living in complexly structured rock pool habitats developed larger telencephalons than their sand-dwelling counterparts, and this supports our previous research which demonstrated that the rock pool species Cocos frillgoby and Krefft's goby have greater spatial cognitive abilities and are capable of processing higher-level sensory information (i.e. they can use multiple cues to orientate) than sand species [White and Brown, 2014a–c]. The telecephalon has a key role to play in fish cognition: processing and coordinating motor and sensory information. Furthermore, for rock pool species, having a larger telencephalon might provide the necessary machinery for more sophisticated orientation and learning (i.e. to find and relocate food, mates and shelter) in complex 3-dimensional habitats. Studies by Van Staaden et al. [1995] and Huber et al. [1997] also found larger telencephalons in cichlid species living in more spatially complex habitats created by shallow rock and vegetation compared to those species living in mid-water. A number of studies have provided evidence for the telencephalon's involvement in various complicated behaviours such as spatial learning [Salas et al., 1996a, b; López et al., 2000], avoidance responses [Portavella et al., 2003, 2004], territoriality, shoaling [Shinozuka and Watanabe, 2004; Martín et al., 2011] and reproductive behaviours such as mating strategy, courtship, nest building and parental care [Overmeir and Gross, 1974; Koyama et al., 1984; Pollen et al., 2007; Broglio et al., 2011].

The optic tectum is both a primary sensory visual structure and a multimodal sensory structure that provides a body-centred framework for multisensory and sensory-motor integration [Stein and Meredith, 1993; Sparks, 2002]. In this study, the optic tectum was the largest brain structure observed in all goby species, on average accounting for 29% of the total brain volume. Evidently vision is a highly developed sense in shallow-water fish. We found clear patterns in optic tectum size that were associated with habitat type; the largest optic tecta were observed in sand-dwelling species (Hoese's sandgoby and eastern longfin goby). These sand-dwelling species have very large eyes perched high on their heads and are assumed to rely heavily on vision to forage effectively and avoid predation [Kuiter, 1996; Hoese, 1998]. The epipelagic zone is one of the clearest aquatic habitats in the ocean [Warrant and Locket, 2004]. Thus, it would make sense that gobies living in this zone, an essentially open habitat with little physical complexity, would invest more brain tissue in visual processing structures that will ultimately help them catch their agile prey and avoid potential danger. An enlarged optic tectum has been correlated with increased swimming speeds in teleost fish that utilize motile prey [Uchihashi, 1953; Huber et al., 1997].

Our previous studies on the sand species, i.e. eastern longfin goby and Hoese's sandgoby, demonstrated that these fish rely heavily on body-centred orientation methods and are also prone to using global cues to orientate [White and Brown, 2014a–c]. Furthermore, sand species live in an unstable and constantly changing homogenous environment where stable landmark cues are not present, thus developing a larger optic tectum that provides a greater capacity for body-centred orientation, and sensory processing is highly advantageous.

One interesting result emerging from this study is that sand gobies have a larger hypothalamus than rock pool-dwelling species. In particular, the hypothalamus appears enlarged in eastern longfin gobies. The hypothalamus controls food intake [Roberts and Savage, 1978], daily cycles in physiological state and behaviour, and many of the neuroendocrine pathways that underlie mating behaviours such as pheromone production, dominance, territoriality and courtship behaviours [Francis et al., 1993; Hofmann and Fernald, 2000; Pollen et al., 2007]; it also receives olfactory impulses from the medial forebrain bundle and telencephalon [Schnitzlein, 1964]. It has been shown in many goby species that pheromones play a vital role in distinguishing between reproductive and non-reproductive conspecifics [Zielinski et al., 2003; Corkum et al., 2006], initiating courtship behaviours [Tavolga, 1956] and synchronizing spawning interactions [MacInnis and Corkum, 2000; Gammon et al., 2005]. Extensive research on the frillfin goby *(B. soporator),* for example, has revealed that ovarian pheromone production by female frillfin gobies has been shown to cause courtship behaviours in males, even if the female is not present [Tavolga, 1956]. It is possible that pheromones may also play an important role in enabling Hoese's sandgobies and eastern longfin gobies to locate reproductive conspecifics in their vast sandy habitat and this may well have favoured morphological adaptations (i.e. related to pheromone production and interpretation) that resulted in an increased hypothalamic volume. Studies on lekking cichlids have shown that certain hypothalamic cell types are enlarged in reproductively mature and territorial individuals and that polygamous males have a larger hypothalamus [Francis et al., 1993; Hofmann and Fernald, 2000; Pollen et al., 2007]. Although these parallels are interesting, it has yet to be proven whether these differences can regulate the volume of the whole hypothalamus.

While we found no differences between sand- and rock pool-dwelling species in the relative size of their cerebellum and dorsal medulla, we did identify differences between species. Cocos frillgobies had both the largest cerebellum and the smallest dorsal medulla. Historically, the cerebellum has been labelled as the control centre of movements in fishes; its size has been correlated with posture, locomotion and other motor associated elements of behavior [Bauchot et al., 1977; New, 2001]. However, an increasing amount of experimental evidence indicates that the cerebellum also plays a role in fish learning, memory and spatial cognition [Rodríguez et al., 2005; Durán et al., 2014]. Previous comparative studies on cichlids have found that piscivorous species had larger cerebella than cichlids feeding on slow-moving or stationary prey [Huber et al., 1997]. In contrast, the dorsal medulla controls operations of the inner organs (i.e. blood pressure, heart rate, digestion and waste disposal) and it is the relay centre for many nerves linking the mid- and forebrain. Here we found that the dorsal medulla was largest in the rock pool-dwelling species Krefft's goby. Previous studies have found links between feeding preferences and dorsal medulla size [Huber et al., 1997; Pollen, 2007], consistent with its role in the processing of taste information. We suggest that species-specific differences in the relative size of these lobes could reflect differences in feeding preferences and may be revealed by future studies examining gut contents.

Although we did not find any differences between sexes for any of the brain lobes studied, we would like to make clear that sex differences should not be entirely discounted in these species, as the sample size was relatively low in this study (i.e. only 5 males and 5 females were used for each species). Future research should utilize larger numbers of males and females in each species and also test greater

numbers of goby species from a wider range of habitats using a formal phylogenetic comparative analysis to explore brain evolution in greater detail for the goby family.

The phylogeny of common gobies in Australia shows that the 2 rock pool-dwelling species are found within the same clade and are thus highly related. The sand gobies, while not occurring in the same clade, are more related to each other than they are to the rock pool species (online suppl. fig.). It is possible, therefore, that the differences we observed in brain morphology between these two habitat types may have occurred once deep in the goby phylogeny. We are not the first authors to link certain phylogenetic clade groups with certain habitat types. Thacker and Roje [2011] grouped the species *Istigobius* and *Favongobius* (referred to as sand species in our study) under the linage name 'lagoon gobies' and grouped *Bathygobius* (referred to as rock pool species in our study) under the linage name 'inshore gobies'. Thus, it is possible that brain morphology and habitat preference of the species studied here have evolved in parallel with their phylogeny.

In conclusion, the results indicate that environmental factors play a key role in brain development in intertidal goby species. The differences in brain structure sizes observed here emphasize the necessity of considering the complex relationship between brain growth and ecological conditions when exploring the evolutionary forces that have led to the large amount of variation in brain and structure sizes that exist in fish and vertebrates in general.

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